THE JOHNS HOPKINS UNIVERSITY

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DEPARTMENT OF BIOLOGY

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Dr. Mark L. Pearson
Director, Molecular Biology
Central Research and Development
E.I. du Pont de Nemours & Co.
Experimental Station E328/258
Wilmington, DE 19898

Dear Mark:

I have recently begun a study of enzymes from extremely thermophilic bacteria in collaboration with Dr. Robert Kelly, Department of Chemical Engineering, The Johns Hopkins University. As an old protein-folder, I find that the phenomenon of enzymatic catalysis at 100°C is quite astounding. There must be a very unusual set of intramolecular interactions that only begin to act cooperatively at temperatures above 70° or 80° and that can stabilize an active center under such conditions. The thermophilic enzymes that have been studied to date, which include proteases, DNA polymerase, and sulphur metabolizing systems show essentially no activity at temperatures below 60° or 70° and suddenly, begin to carry out their proper function as the temperature nears that at which the organisms normally live. The bacteria that we are using grow at 90° to 105° and the rate of catalysis at these temperatures seems to be five- to ten-fold greater than that of analogous enzymes in mesophiles at their optimal temperatures of around 25° to 40°.

I am writing to you because it struck me that some of these catalysts might be of value in the processes employed in the pharmaceutical industry, and perhaps even in other aspects of industrial chemistry. I know that at present, Genentech is using a DNA polymerase from thermus aquaticus for high-speed DNA polymerization, with the advantage that the high temperature keeps the template DNA in a random uncoiled form, and the enzyme itself is stable. Proteases are also being used, I believe, in large-scale hydrolysis for the preparation of hydrolysates of nutritional or agricultural value. Bob Kelly, who I mentioned above, is particularly concentrating on thermophilic enzymes of sulphur metabolism which reduce to produce H_2S or oxidize to produce sulphate, and these systems might well become of major value in the desulfurization of coal.

At the moment, we are combining affinity chromatography and HPLC techniques to study several enzymes. Thermophilic galactosidase, for example, will stick to a silica-based affinity column only at temperatures high enough to permit formation of the proper active site in the protein. At lower temperatures, the enzyme detaches and is eluted, and this reversible process can be regulated by controlling the

temperature of the HPLC column. Our work at the moment is exploratory in the sense that we are looking at half a dozen enzyme systmes to establish a standard procedure for affinity column purification and subsequent attachment of purified enzymes to affinity columns for on-line use in the pertinent process.

Since I feel that some of our work might be of interest in terms of an ultimately patentable procedure that would permit efficient, high-speed chemical conversions, I thought I might as well drop you a note so that you and your colleagues could consider such a possibility. If there is interest, it would be particularly valuable to know what kinds of processes and chemical steps are critical in your manufacturing activities. This would permit us to home in on just not any old enzyme, but those that would be of specific use.

As you will sense from what I have already written, I am looking for sources of support for such collaborative work under the usual patent agreement systems that exist between industry and academia. I am leaving for a two-week trip on my boat on the 24th of July and will be back by the beginning of the second week in August. I would greatly appreciate your comments on the possiblilty of some mutually advantageous agreement.

Sincerely yours,

Chris

Christian B. Anfinsen

CBA:djh

(Pete Knight suggested that you would be the right person to write to at Dupont.)